Translation

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/FR2004/000354	International filing date *(day/month/year)* 17.02.2004	Priority date *(day/month/year)* 18.02.2003
International Patent Classification (IPC) or national classification and IPC C12N15/01 , C12N9/10, C12P13/06, C12P13/08, C12P13/12		
Applicant METABOLIC EXPLORER		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____ , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FR2004/000354

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rule 12.3 and 23.1(b))

☐ publication of the international application (Rule 12.4)

☐ international preliminary examination (Rule 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-79 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

nos. 1-37 as originally filed/furnished

nos.* _____ as amended (together with any statement) under Article 19

nos.* _____ received by this Authority on _____

nos.* _____ received by this Authority on _____

☒ the drawings:

sheets 1/2-12/12 as originally filed/furnished

sheets* _____ received by this Authority on _____

sheets* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. III **Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 1-4, 7-35 (in part, where applicable); 5, 6, 36, 37 (in full)

because:

☐ the said international application, or the said claims Nos. _____
relate to the following subject matter which does not require an international preliminary examination (*specify*):

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. see below
are so unclear that no meaningful opinion could be formed (*specify*):

11-28, 35 (in part, where applicable); 29-34 (in full)

See supplemental box

☒ the claims, or said claims Nos. 11-28, 35 (in part, where applicable); 29-34 (in full) are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 1-4, 7-35 (in part, where applicable); 5, 6, 36, 37 (in full)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted the claims nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

   See supplemental box

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-4, 7-35 (in part, where applicable)

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	26	YES
	Claims	1-4, 7-25, 27, 28, 35	NO
Inventive step (IS)	Claims	26	YES
	Claims	1-4, 7-25, 27, 28, 35	NO
Industrial applicability (IA)	Claims	1-4, 7-28, 35	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

1. Reference is made to the following documents in the present notification:

D1: WO 93/17112 A (GENENCOR INT) 2 September 1993 (1993-09-02)

D2: DUCHANGE N ET AL: "E. coli metB and metL (5' end) genes coding for cystathione gamma-synthase and aspartokinase II-homoserine dehydrogenase II" EMBL, 13 June 1985 (1985-06-13), XP002274156

D3: KAWASHIMA T ET AL: "Cystathionine beta lyase / O-succinylhomoserine lyase" EMBL, 1 October 2001 (2001-10-01), XP002274157

2. NOVELTY (PCT Article 33(2)) AND INVENTIVE STEP (PCT Article 33(3))

2.1 Document D1 describes (cf. pages 1, 2, 5 (point 5) and examples 1 and 3) a method for preparing evolved microorganisms (*E. coli, C. glutamicum and B. flavum*) to enable modification of the methionine biosynthesis pathway, characterised in that it comprises the steps of (a) providing a

modified microorganism by genetically modifying the cells of a starting microorganism so as to inhibit the production of a metabolite (homoserine) when the microorganism is cultured on a predetermined medium, whereby the growth capacity of the microorganism is adversely affected; (b) culturing the previously modified microorganisms obtained on said medium defined to cause evolution thereof (the medium contains glucose, soybean hydrolysate and inorganic nutrients, and the co-substrate enabling evolution is methyl mercaptan or H_2S); and (c) selecting cells having modified microorganisms capable of developing on the predetermined medium with the co-substrate. The method comprises an additional step (a1) of inserting at least one heterologous gene coding for a heterologous protein, which heterologous gene is intended to enable the evolution of a new metabolic pathway prior to step (b), i.e. a step of inserting genes coding for cystathione gamma-synthase and O-acyl-L-homoserine sulfhydrolase. Protein evolution enables the inhibited metabolic pathway (homoserine) to be replaced by a new metabolic pathway (methionine). It follows that the subject matter of claims 1 to 4 and 7 to 14 is anticipated by document D1. Furthermore, D1 shows such a gene coding for such a modified protein having "methionine synthase" activity and selected from cystathione gamma-synthases and O-acyl-L-homoserine sulfhydrolases, as well as the use of such a microorganisms or such a protein in a biotransformation method, i.e.

the preparation of methionine. Consequently, the subject matter of claims 15 to 17 and 35 is anticipated by document D1.

2.2 The terms "modified" "modification(s)", "evolved" and "corresponding", as used in the claims, are vague, undefined and equivocal and thus cast doubt on the meaning of the technical features to which they refer and on the scope of the claims. It follows that the subject matter of the claims has not been clearly defined (PCT Article 6), and that the novelty of the claims is affected.

2.3 The indication "K183" does not appear to add an essential technical feature to the definition of claim 12.

2.4 Claims 18 to 25, 27 and 28 do not contain any features which, when combined with the features of any one of the claims to which they refer, comply with the requirements of novelty and inventive step of the PCT (PCT Article 33(2) and (3)). Document D2 (cf. the whole document) shows an "unmodified" cystathione gamma-synthase that is 100 % identical to the cystathione gamma-synthase sequence of E. coli K12 shown in SEQ ID NO 6. Document D3 (cf. the whole document) shows an "evolved" or "modified" enzyme including the amino acid sequence AASLGGVES in the C-terminal portion thereof, which sequence "matches" residues 324 to 332 of the sequence of E. coli cystathione gamma-synthase shown in SEQ ID NO 8.

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

2.5 The combination of features in claim 26 is not found in or obvious from the prior art because it is not obvious for a person skilled in the art to arrive at a cystathione gamma-synthase having "methionine synthase" activity and including the amino acid sequence shown in SEQ ID NO 8.

2.6 The present application fails to comply with the requirements of PCT Article 33(1) since the subject matter of claims 1 to 4, 7 to 25, 27, 28 and 35 does not meet the requirement of novelty defined in PCT Article 33(2) and does not involve an inventive step as defined in PCT Article 33(3).

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The sequence listing in the description, pages 1-14, as originally filed

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Box III

1. Claims: 1-4, 7-35 (in part, where applicable), 5, 6, 36, 37 (in full) (cf. Box IV: Lack of unity of invention: invention 1):
A method for preparing evolved microorganisms to enable modification of a methionine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

2. The present claims 11 to 35 relate to products defined by reference to a desirable property or characteristic, namely the method by means of which they can be prepared and/or the fact that the enzyme in question has "modified methionine synthase" activity.

The claims cover all of the products that have this property or characteristic, whereas the application provides support (PCT Article 6) and disclosure (PCT Article 5) for only a very limited number of such products. In the present case, the claims lack support and the application lacks disclosure to such an extent that it is impossible to carry out a meaningful search covering the entire range of protection sought. Independently of the reasons given above, the claims also lack clarity (PCT Article 6). Indeed, an attempt has been made to define the product in terms of the

Supplemental Box

method by means of which they can be prepared and/or the fact that the enzyme in question has "modified methionine synthase" activity. This lack of clarity is, again, such that it is impossible to carry out a meaningful search covering the entire range of protection sought. Therefore, the search was directed only to the parts of the claims of which the subject matter appears to be clear, supported and sufficiently disclosed, namely the parts that relate to cystathione gamma-synthase mutation E325A (cf. claims 25 and 26) and clone K183 (cf. claim 12).

Box IV

The various groups of inventions are as follows:

1. Claims 1 to 4 and 7 to 35 (in part, where applicable).

   A method for preparing evolved microorganisms to enable modification of a methionine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

2. Claims 1 to 4 and 7 to 35 (in part, where applicable).

   A method for preparing evolved microorganisms to enable modification of a cysteine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

3. Claims 1 to 4 and 7 to 35 (in part, where applicable).

   A method for preparing evolved microorganisms to enable modification of a threonine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

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4. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a lysine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

5. Claims 1 to 4 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of a isoleucine biosynthesis pathway, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

6. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of nucleic acid biosynthesis pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

7. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of lipid biosynthesis

pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

8. Claims 1, 2 and 7 to 35 (in part, where applicable).

A method for preparing evolved microorganisms to enable modification of sugar biosynthesis pathways, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

9. Claims 1, 2 and 7 to 35 (in part, where applicable); 5, 6, 36, 37 (in full).

A method for preparing evolved microorganisms to enable modification of the metabolic pathways involved in NADPH consumption, microorganisms, genes and proteins obtained by means of said method, and the use thereof in a biotransformation method.

The above inventions are not so linked as to form a single general inventive concept (PCT Rule 13.1), for the following reasons:

The prior art describes methods for preparing evolved microorganisms to enable modification of metabolic pathways (e.g. nucleic acid biosynthesis pathways and methionine biosynthesis), including the three steps described in claim 1. Document WO

02/083892 (cf. claims 1 to 30) describes an artificial *in vivo* protein evolution method whereby a protein X (e.g. a kinase) can be evolved by complementation of a related protein Y. the mutant protein X has a broader activity than the starting protein (for example, mutants D133E and R104Q of deoxycytidine kinase (DCK) have been obtained, and each of these mutations confers the acquisition of thymidine kinase activity by DCK). Document XP002154849 (cf. the whole document) describes a method for preparing evolved microorganisms including the three steps described in claim 1. Mutant hydantoinase has a reversed enantioselectivity and can be used in the an improved method for producing L-methionine.

In the light of the prior art, the problem addressed by the present application is that of providing alternative methods for preparing evolved microorganisms to enable alternative modification of metabolic pathways, including the three steps described in claim 1. Solutions 1 to 9 to said problem amount to providing methods for preparing evolved microorganisms to enable modification of a metabolic pathway relating to:

(1) methionine biosynthesis;
(2) cysteine biosynthesis;
(3) threonine biosynthesis;
(4) lysine biosynthesis;
(5) isoleucine biosynthesis;
(6) nucleic acid biosynthesis;
(7) lipid biosynthesis;

Supplemental Box

(8) sugar biosynthesis;

(9) NADPH consumption.

Given that the methods for preparing evolved microorganisms to enable alternative modification of metabolic pathways, including the three steps described in claim 1, are described in the prior art (cf. WO 02/83892 and XP002154849), as a result of the essential technical differences between said solutions, and in view of the fact that it has been impossible to determine any other feature which might be considered to be a special technical feature in the light of the prior art the Search Division is of the opinion that no single general inventive concept covers the plurality of solutions proposed in the present application. It follows that the required unity of invention does not exist (PCT Rule 13.1) and since the various inventions do not have a common inventive concept, they are drafted as separate subjects as well as notified (PCT Article 17(3)(a)).